COVID-19 drugs chloroquine and hydroxychloroquine, but not azithromycin and remdesivir, block hERG potassium channels

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Running Title: Effects of four COVID-19 drugs on hERG channels

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Abbreviations: HEK, human embryonic kidney; hERG, human ether-a-go-go related gene; IC_{50}, half maximal inhibitory concentration; I_{hERG}, hERG current; I_{Kr}, rapidly activating delayed rectifier potassium current; I_{Ks}, slowly activating delayed rectifier K^{+} current; LQTS, long QT syndrome; MEM, minimum essential medium; QT_{c}, corrected QT interval; V_{1/2}, voltage of half maximal activation. CQ, chloroquine; HCQ, hydroxychloroquine; AZ, azithromycin; RDV, remdesivir.

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ABSTRACT

Drug-induced long QT syndrome (LQTS) is an established cardiac side effect of a wide range of medications, which represents a significant concern for drug safety. The rapidly (I_{K_r}) and slowly activating delayed rectifier K^+ currents (I_{K_s}) mediated by channels encoded by the human ether-a-go-go-related gene (hERG) and KCNQ1+KCNE1, respectively, are two main currents responsible for ventricular repolarization. The common cause for drugs to induce LQTS is through impairing the hERG channel. For the recent emergence of COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), several drugs have been investigated as potential therapies, however there are concerns about their QT prolongation risk. Here we study the effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on hERG channels. Our results showed that while chloroquine acutely blocked hERG current (I_{hERG}) with an IC_{50} of 3.0 µM, hydroxychloroquine acutely blocked I_{hERG} 8-fold less potently, with an IC_{50} of 23.4 µM. Azithromycin and remdesivir did not acutely affect I_{hERG}. When these drugs at 10 µM were added to the cell culture medium for 24 h, remdesivir increased I_{hERG} by two-fold, which was associated with an increased mature hERG channel expression. In addition, these four drugs did not acutely or chronically affect KCNQ1+KCNE1 channels. Our data provide insight into COVID-19 drug-associated LQTS and cardiac safety concerns.
SIGNIFICANCE STATEMENT

This work demonstrates that among off-label potential COVID-19 treatment drugs chloroquine, hydroxychloroquine, azithromycin, and remdesivir, the former two drugs block hERG potassium channels while the latter two drugs do not. All four drugs do not affect KCNQ1+KCNE1. As hERG and KCNQ1+KCNE1 are two main K$^+$ channels responsible for ventricular repolarization, and most drugs that induce long QT syndrome (LQTS) do so by impairing hERG channels, our data provide insight into COVID-19 drug-associated LQTS and cardiac safety concerns.
INTRODUCTION

The pandemic of the novel coronavirus disease of 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents an unprecedented healthcare challenge. Given the urgently needed but limited treatment options for COVID-19, off-label use of chloroquine and hydroxychloroquine, sometimes with azithromycin, is under investigation (Kamp et al., 2020). As well, the antiviral drug remdesivir is being actively investigated (Spinner et al., 2020). While effectiveness of these drugs have been reported in various clinical trials (Gautret et al., 2020; Monti et al., 2020), concerns about their proarrhythmic effects associated with prolonged QT intervals have been raised (Ramireddy et al., 2020; Mercuro et al., 2020; Hooks et al., 2020; Gerard et al., 2020; Szekely et al., 2020; Maraj et al., 2020; Chorin et al., 2020).

An abnormal prolongation of QT interval in electrocardiogram (ECG) is defined as long QT syndrome (LQTS) which can develop into fatal ventricular arrhythmia Torsade de Pointes and lead to sudden cardiac death (Schwartz et al., 1993; Curran et al., 1995; Roden et al., 1996; January et al., 2000; Keating and Sanguinetti, 2001). Inherited LQTS occurs in 1 out of ~2000 individuals due to mutations in various ion channels and adaptor proteins in the heart (Schwartz et al., 2009; Schwartz et al., 2012; Zaklyazminskaya and Abriel, 2012). When LQTS occurs due to an environmental stressor, such as specific drug usage or hypokalemia, it is termed acquired LQTS. Drug-induced LQTS is a side-effect of a surprisingly wide spectrum of medications; it represents the single most common cause of the withdrawal or restriction of the use of prescription drugs (Roden, 2004), and a significant concern during drug development as many lead compounds with therapeutic potential are dropped from further development because of this risk (Finlayson et al., 2004; Shah, 2005). While many types of ion channels contribute to the
cardiac action potential, most drugs that cause LQTS do so by acting on the cardiac rapidly activating delayed rectifier K$^+$ current ($I_{Kr}$) (Roden et al., 1996; Mitcheson et al., 2000; January et al., 2000; Keating and Sanguinetti, 2001; Roden, 2004; Perrin et al., 2008) which is conducted through channels encoded by human ether-a-go-go related gene (hERG) (Sanguinetti and Tristani-Firouzi, 2006; Sanguinetti et al., 1995; Trudeau et al., 1995). Experimentally, the current of hERG channels expressed in cell lines remarkably resembles $I_{Kr}$ in the cardiac myocytes (Guo et al., 2007; Guo et al., 2009). hERG current ($I_{hERG}$) is critical for repolarization of the cardiac action potential (Curran et al., 1995; Keating and Sanguinetti, 2001). A reduction of $I_{hERG}$ prolongs ventricular action potential duration which is the cellular basis for QT intervals in ECG recordings.

Chloroquine has been used for malaria treatment and prophylaxis (Coban, 2020). During the Severe Acute Respiratory Syndrome (SARS) pandemic in 2002-2004, chloroquine was reported to inhibit SARS coronavirus (SARS-CoV-1) (Keyaerts et al., 2004). It has been added to the list of trial drugs for the treatment of COVID-19 (Hu et al., 2020b). Hydroxychloroquine is a derivative of chloroquine with an additional hydroxyl group. It is less toxic than chloroquine, and has been used to treat autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, and is also considered as a candidate drug to treat COVID-19 (Liu et al., 2020). Experimentally, both chloroquine and hydroxychloroquine are reported to efficiently inhibit SARS-CoV-2 infection in vitro (Liu et al., 2020). A clinical trial indicated an association of hydroxychloroquine treatment with viral load reduction/disappearance in COVID-19 patients, and this association is reinforced by the addition of the macrolide antibiotic azithromycin (Gautret et al., 2020). On the other hand, it was reported recently that the effectiveness of hydroxychloroquine on COVID-19 warrants further investigation (Monti et al., 2020).
Nevertheless, chloroquine and hydroxychloroquine remain treatment options to fight COVID-19 (Colson et al., 2020). However, COVID-19 patients receiving chloroquine or hydroxychloroquine as a treatment were associated with a high risk of corrected QT interval (QTc) prolongation (Ramireddy et al., 2020; Hooks et al., 2020; Gerard et al., 2020; Szekely et al., 2020), and those receiving both hydroxychloroquine and azithromycin were associated with greater changes in QTc (Chorin et al., 2020; Mercuro et al., 2020). The antiviral agent remdesivir (Veklury®, Gilead Sciences) has demonstrated potent anti-viral activity against SARS-CoV-2 in preclinical studies, and has emerged as a drug for COVID-19 treatment (Lamb, 2020). The risk of remdesivir for inducing LQTS is unknown.

In the present study, in order to obtain insight into potential QT prolongation induced by COVID-19 drugs, we investigated the effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on hERG channels stably expressed in a hERG-HEK cell line. We also examined their effects on KCNQ1+KCNE1 channels which mediate the slowly activating delayed rectifier K\(^+\) current (I\(_{Ks}\)) (Sanguinetti et al., 1996; Barhanin et al., 1996). The clinical implications of our findings are discussed.

**MATERIALS AND METHODS**

**Molecular Biology**

The HEK293 cell line stably expressing hERG channels (hERG-HEK cells) was provided by Dr. Craig January (University of Wisconsin, Madison) (Zhou et al., 1998b). Human KCNQ1 and KCNE1 cDNAs were provided by Dr. Michael Sanguinetti (University of Utah) (Sanguinetti et al., 1996). HEK293 cell line stably expressing KCNQ1+KCNE1 (KCNQ1+KCNE1-HEK cells) was created using plasmid transfection followed by G418 (1 mg/mL) selection (Guo et al.,
hERG-HEK and KCNQ1+KCNE1-HEK cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, nonessential amino acids (1×), sodium pyruvate (1 mM), and G418 (0.4 mg/mL). For electrophysiological recordings, the cells were collected from the culture dish and kept for up to 4 h in vials with MEM at room temperature until use.

**Electrophysiological recordings**

The whole-cell voltage-clamp method was used to record hERG current ($I_{\text{hERG}}$) and KCNQ1+KCNE1 current ($I_{\text{KCNQ1+KCNE1}}$) (Guo *et al.*, 2011). Pipettes were pulled from thin-walled borosilicate glass (World Precision Instruments, Sarasota, CA) using a micropipette puller (P-1000, Sutter Instrument, Novato, CA) and polished using a Micro Forge (MF-830, Narishige, Tokyo, Japan) to achieve a resistance of ~2.0 MΩ when filled with solution. Axopatch 200B amplifier, Digidata 1440A digitizer, and pCLAMP10.7 software (Molecular Devices, San Jose, CA) were used for data acquisition and analysis. The pipette solution contained (in mM) 135 KCl, 5 EGTA, 5 MgATP, and 10 HEPES (pH 7.2 with KOH). The bath solution contained (in mM) 5 KCl, 135 NaCl, 2 CaCl$_2$, 1 MgCl$_2$, 10 glucose, and 10 HEPES (pH 7.4 with NaOH). Recordings were carried out at room temperature (22±1°C).

**Western blot analysis**

Following treatments, hERG-HEK cells were collected in ice-cold phosphate-buffered saline (PBS). The whole-protein samples were made by lysing cells using sonication in ice-cold radioimmunoprecipitation assay lysis buffer supplemented with 1 mM PMSF (phenylmethylsulfonyl fluoride) and 4% protease inhibitor cocktail. A DC Protein Assay Kit (Bio-Rad, Hercules, CA) was used to determine protein concentration of samples. 15 µg of protein in 50 µL Laemmli loading buffer containing 5% β-mercaptoethanol was boiled for 5 min.
and loaded on 8% SDS polyacrylamide electrophoresis gels for separation for 3 h. Separated proteins were transferred onto polyvinylidene difluoride membranes overnight in a cold room (4°C). The membranes were blocked for 1 h using 5% non-fat milk in TBST (Tris-buffered Saline with 1% Tween 20). The blots were probed for 1 h with anti-hERG primary antibodies in 5% non-fat milk in TBST and then incubated with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies in TBST for 1 h. Primary and secondary antibodies were washed using TBST for 5 min each for three times. Detection of β-actin was performed for loading control. The blots were visualized with Fuji medical X-ray films (Minato, Tokyo, Japan) using an enhanced chemiluminescence detection kit (GE Healthcare, Little Chalfont, United Kingdom). Protein band sizes were determined by loading BLUeye Prestained protein ladder (GeneDirex, Taiwan) on each gel. The hERG channel protein displays two distinct bands at molecular masses of 135 and 155 kDa. The 135-kDa band corresponds to the immature core-glycosylated form of the channel located in the endoplasmic reticulum, whereas the 155-kDa band is the fully-glycosylated form of the channel present on the plasma membrane. The 155-kDa mature form of the channel is responsible for generating \( I_{\text{hERG}} \) (Zhou et al., 1998a; Guo et al., 2007; Guo et al., 2009; Lamothe et al., 2016; Lamothe et al., 2018). For quantification of Western blot data in Fig. 5B and C, band intensities of hERG proteins in each treatment were normalized to their respective actin loading control and then to the control (CTL, or 0) in the same gel, and expressed as relative values.

**Drugs and reagents**

Hydroxychloroquine sulfate and azithromycin were purchased from Cayman Chemical Company (Ann Arbor, MI). Chloroquine disulfate was purchased from Sigma-Aldrich (St. Louis, MI). Remdesivir was obtained from Tocris Bioscience (Bristol, United Kingdom).
Hydroxychloroquine sulfate and chloroquine disulfate were diluted in double-distilled water, azithromycin was diluted in ethanol and remdesivir was diluted in DMSO to stock solutions of 10 and 50 mM, and stored at −20°C. To study drug block, the drugs were diluted in bath solution and used for patch-clamp experiments within 8 h. To study chronic drug effect, drugs were diluted in MEM at desired concentrations. To exclude any potential vehicle-related effects on channel activities in studies of drug-channel interaction, corresponding amount of vehicles was applied to control groups. Specifically, a highest concentration of 0.2% DMSO or 0.1% ethanol was used in control cells for remdesivir (90 µM) or azithromycin (50 µM), which had no effect on either I_{hERG} or I_{KCNQ1+KCNE1}, acutely or chronically. MEM, FBS, G418 (Geneticin), non-essential amino acids, and sodium pyruvate were purchased from Thermo Fisher Scientific (Waltham, MA). Mouse anti-actin primary antibody, EGTA, HEPES, glucose, and electrolytes were purchased from Sigma-Aldrich (St. Louis, MO). Mouse anti-hERG (F-12) primary antibody was purchased from Santa Cruz Biotechnology (Dallas, TX). Horse anti-mouse HRP-conjugated secondary antibody was purchased from Cell Signaling Technology (Danvers, MA). The F-12 primary antibody was used in a 1:1000 dilution, anti-actin primary antibody was used in a 1:2000 dilution, and anti-mouse secondary antibody was used in a 1:20000 dilution.

**Statistical analysis**

Data are expressed as the mean ± the Standard Deviation (SD) or box plots including mean, median and individual data points. A one-way ANOVA with Dunnett’s post-hoc test or 2-tailed Student’s t-test were used to determine statistical significance between the control and test groups. For normalized data, a Wilcoxon matched pairs test was used to compare currents with each drug to control currents. Statistical analysis was performed with GraphPad Prism 8.4. A P-value of 0.05 or less was considered statistically significant.
RESULTS

Chloroquine and hydroxychloroquine, but not azithromycin and remdesivir, acutely block hERG currents

Whole cell voltage-clamp was used to record $I_{\text{hERG}}$ in hERG expressing HEK 293 cells. $I_{\text{hERG}}$ was elicited using the protocol shown in the top of Fig. 1. The holding potential was -80 mV, which was then depolarized to +50 mV for 4 s, before repolarizing to -50 mV for 5 s. Activation and inactivation of the hERG channel occurs during the depolarizing step, followed by rapid recovery to the open channel state with slow deactivation during the repolarizing step at -50 mV. This rapid recovery to the open channel state results in the characteristic tail current of the hERG channel, which is measured to quantify the amplitude of $I_{\text{hERG}}$ as it represents the maximal conductance (Spector et al., 1996). The voltage protocol was delivered every 15 s and $I_{\text{hERG}}$ was recorded before (control, black traces) and after drug application to bath solution (treatment, red traces) in the same cells. To compare the effects of the four drugs on $I_{\text{hERG}}$, a concentration of 10 µM of each drug was examined. Chloroquine decreased $I_{\text{hERG}}$ by more than half, while hydroxychloroquine slightly decreased $I_{\text{hERG}}$. However, neither azithromycin nor remdesivir affected $I_{\text{hERG}}$ (Fig. 1).

To determine the concentration dependent effects on $I_{\text{hERG}}$, following recording of control currents, a range from 0.5 to 50 µM drugs were applied consecutively to the same cells during patch-clamp recordings. The peak tail current amplitude at each concentration was normalized to the control current of the cell, 13-19 cells were examined, and summarized data were used to construct the concentration-response relationships. Data were fitted to the Hill equation to obtain the concentration for half maximal inhibition ($IC_{50}$). Chloroquine blocked $I_{\text{hERG}}$ with an $IC_{50}$ of
3.0±0.3 µM with a Hill coefficient of 0.9±0.1 (n=6-19 for each concentration). Hydroxychloroquine blocked I_{hERG} with an IC<sub>50</sub> of 23.4±7.9 µM with a Hill coefficient of 0.8±0.2 (n=7-13 for each concentration). Thus, while hydroxychloroquine does block I_{hERG}, it does so with a potency 8-fold less than chloroquine. In addition, neither azithromycin nor remdesivir affected I_{hERG} at 10 or 50 µM (Fig. 2).

To study the effects of chloroquine on the activation-voltage relationships, I_{hERG} was recorded by depolarizing cells from the holding potential of -80 mV to voltages between -70 and 70 mV in 10 mV increments followed by a repolarizing step to -50 mV. The pulse currents measured at the end of 4-s depolarizing steps and the peak tail currents measured upon the repolarization to -50 mV, normalized to their respective maximal amplitude in control, were plotted against depolarizing (activation) voltages. Chloroquine (10 µM) blocked both pulse and tail currents (Fig. 3). The tail current-voltage relationships were fitted to the Boltzmann equation to obtain the half maximal activation voltages (V<sub>1/2</sub>). Chloroquine shifted the V<sub>1/2</sub> to negative voltages by 6.4 mV (−1.0±2.8 mV in control; −7.4±3.3 mV with chloroquine, P<0.05) without affecting the slope factor (7.5±0.5 in control, 7.2±0.3 with chloroquine, P>0.05, n=7).

Treatment of hERG-HEK cells for 24 h with remdesivir, but not chloroquine, hydroxychloroquine or azithromycin, increases I_{hERG} and mature hERG expression

Drugs may affect hERG function not only by interfering with channel function but also by altering channel expression levels (Ficker et al., 2004; Kuryshov et al., 2005; Guo et al., 2007). To determine whether chronic exposure of hERG channels to these drugs affect I_{hERG}, hERG-HEK cells were cultured for 24 h with 10 µM of chloroquine, hydroxychloroquine, azithromycin or remdesivir, respectively. Once cultured, cells were collected and subject to whole-cell voltage clamp. Twenty-four h treatment with 10 µM remdesivir increased I_{hERG} by
about 2-fold (Fig. 4). When tail currents were plotted against depolarizing voltages and data were fitted to Boltzmann equation, remdesivir increased $I_{\text{hERG}}$ without affecting $V_{1/2}$ ($V_{1/2}$: 2.0±1.1, slope factor: 6.6±0.2 in control; $V_{1/2}$: 1.3±2.1, slope factor: 7.3±0.2 with remdesivir, n=10, $P>0.05$).

To investigate the concentration-dependent effects of chronic treatment with remdesivir on $I_{\text{hERG}}$, hERG-HEK cells were cultured with remdesivir at 0 (control), 1, 3, 10, 30 and 90 µM for 24 h. However, a clear sigmoidal concentration-dependent effect was not observed (Fig. 5A). While an increase in $I_{\text{hERG}}$ was observed at 10 µM, 30 or 90 µM did not cause additional increases but instead decreased $I_{\text{hERG}}$ slightly compared to 10 µM. Chronic treatments with drugs may lead to a changed hERG protein expression (Kuryshev et al., 2005; Guo et al., 2007; Lamothe and Zhang, 2013; Wang et al., 2014; Sutherland-Deveen et al., 2019). To investigate whether an altered hERG protein expression is associated with the changes in $I_{\text{hERG}}$, we performed Western blot analysis. As shown in Fig. 5B, after a 24 h culture at 10 µM, chloroquine, hydroxychloroquine, and azithromycin had no effect on hERG expression. However, remdesivir increased the 155-kDa hERG band density, which may underlie $I_{\text{hERG}}$ increase. Subsequently, hERG-HEK cells were treated with varying concentrations of remdesivir for 24 h and subject to Western blot analysis to determine if the increase of mature hERG expression was concentration dependent. As shown in Fig. 5C, remdesivir increased 155-kDa hERG expression in a concentration-dependent manner although the increase at the highest concentration (90 µM) appeared less prominent than that of 30 µM.

**Chloroquine, hydroxychloroquine, azithromycin and remdesivir do not affect $I_{\text{KCNQ1+KCNE1}}$**

In addition to $I_{\text{Kr}}$/hERG, $I_{\text{Ks}}$, which is encoded by KCNQ1 and KCNE1, is another important current for cardiac repolarization (Sanguinetti et al., 1996; Barhanin et al., 1996). We recorded
KCNQ1+KCNE1 current ($I_{\text{KCNQ1+KCNE1}}$) from HEK cells that stably express both KCNQ1 and KCNE1. After recording control currents, drugs were added to the bath solution, and currents were recorded again in the same cells. For analysis, currents in the presence of drugs are normalized to their respective controls and presented as relative current for each drug. Chloroquine, hydroxychloroquine, azithromycin or remdesivir did not acutely affect $I_{\text{KCNQ1+KCNE1}}$ (Fig. 6). To investigate whether chronic treatments with these drugs affect $I_{\text{KCNQ1+KCNE1}}$, KCNQ1+KCNE1-HEK cells were cultured in control or with 10 µM of chloroquine, hydroxychloroquine, azithromycin or remdesivir for 24 h and then examined with whole-cell patch clamp recordings using the voltage protocol shown in Fig. 4A. Current amplitudes upon 50 mV depolarizing step in each group were demonstrated in box plots. None of the four drugs chronically affected $I_{\text{KCNQ1+KCNE1}}$ (Fig. 7).

DISCUSSION

In the present study, we investigated the acute and chronic effects of chloroquine, hydroxychloroquine, azithromycin, and remdesivir on two primary repolarizing K$^+$ currents, $I_{\text{hERG}}$ and $I_{\text{KCNQ1+KCNE1}}$. Our data showed that chloroquine blocked $I_{\text{hERG}}$ with an $IC_{50}$ of 3.0 µM, while hydroxychloroquine blocked $I_{\text{hERG}}$ with a potency 8-fold less, with an $IC_{50}$ of 23.4 µM. Neither azithromycin nor remdesivir blocked $I_{\text{hERG}}$ at concentrations up to 50 µM. When drugs were added to the cell culture medium for 24 h, chloroquine, hydroxychloroquine and azithromycin did not affect $I_{\text{hERG}}$ and hERG expression levels. However, remdesivir at 10 µM chronically increased $I_{\text{hERG}}$ by 2-fold, which was associated with an increased expression of the mature hERG proteins. Furthermore, none of these drugs acutely or chronically affected $I_{\text{KCNQ1+KCNE1}}$. These findings are summarized in Fig. 8.
Since reliable information on drug concentrations or doses for treating COVID-19 is lacking, doses that proved effective and safe in the treatment of other diseases such as malaria can be considered. For chloroquine, peak serum concentrations of 0.82 µM (263 ng/mL) and 1.37 µM for malaria treatment (Walker et al., 1983; Rombo et al., 1987) was reported and a target plasma concentration not above 2.5 or 3.0 µM has been proposed (Smit et al., 2020; White et al., 2020). For hydroxychloroquine, the average serum/plasma hydroxychloroquine concentrations for patients with systemic lupus erythematosus were found below 480 ng/mL (1.43 µM) which is two-fold of the EC₅₀ of 0.72 µM obtained in an in vitro study targeting SARS-CoV-2 with hydroxychloroquine for 48 h (Carlsson et al., 2020; Balevic et al., 2020; Yao et al., 2020). For azithromycin, plasma/serum concentration was 240-650 ng/ml (0.32-0.87 µM) during the treatment of lung infection (Jeong et al., 2016) and was 565.53 ng/mL (0.75 µM) in a clinical study involving healthy volunteers (Matzneller et al., 2013). For remdesivir, the mean plasma concentration–time profiles after intravenous administration were documented in two critically ill COVID-19 patients who recovered (Tempestilli et al., 2020). In these two patients, 200 mg remdesivir was intravenously administered over 1 h on day 1, followed by one-time 100 mg administration daily for additional 12 days. On Day 3-9, blood samples were collected immediately after, at 1 and 24 h after remdesivir administration. It was shown that immediately after remdesivir administration, mean peak serum concentrations for the two patients were 2737 and 3317 ng/mL (4.54 and 5.50 µM). Plasma concentration of remdesivir decayed rapidly; 1 h after infusion, they were 80.7 and 171 ng/mL (0.13 and 0.28 µM), respectively; and 24 h after infusion, they were below the limit of quantification (Tempestilli et al., 2020). It is important to note that drugs may bind to serum proteins, and their free concentrations remain unknown but are expected to be even lower. Thus, it appears that all these drugs can reach single-digit µM
levels in clinical settings. While 10 µM of each of the four drugs was examined during whole-cell voltage clamp recordings, only chloroquine significantly decreased $I_{\text{hERG}}$ (Fig. 1). Chloroquine-induced hERG block was previous reported (Traebert et al., 2004; Sanchez-Chapula et al., 2002).

Our results showed that chloroquine and hydroxychloroquine acutely blocked $I_{\text{hERG}}$ in a concentration dependent manner with hydroxychloroquine 8-fold lesser potency than chloroquine ($IC_{50}$ being 23.4 vs. 3.0 µM). The ~8 fold difference in hERG blocking potency is due to the hydroxyl group which is the only difference between hydroxychloroquine and chloroquine. Molecular mechanisms underlying a hydroxyl addition induced reduction of hERG block by chloroquine warrant further investigation. As an adverse unwanted effect, drug-induced hERG block and potential LQTS represents a significant concern for drug safety (Finlayson et al., 2004; Shah, 2005; Kannankeril et al., 2010). The addition of a hydroxyl to the ethyl group of chloroquine to form hydroxychloroquine which retains drug properties while reducing hERG-blocking potency would be of great interest for drug development.

We also investigated the chronic effects the four drugs on the current and expression of hERG channels, and found that none of them chronically decreased $I_{\text{hERG}}$. However, treatment of cells with 10 µM remdesivir for 24 h increased $I_{\text{hERG}}$ by 2-fold, and an increased channel expression is responsible for increase $I_{\text{hERG}}$ (Fig. 4 and 5). The molecular mechanisms underlying remdesivir-induced increase in $I_{\text{hERG}}$ and hERG protein expression are currently unknown and warrant future investigation. The clinical relevance of remdesivir-mediated increase in $I_{\text{hERG}}$ is also unknown. Some hERG mutations can cause short QT syndrome and arrhythmias due to the increased $I_{\text{hERG}}$ (Brugada et al., 2004; Schimpf et al., 2005). The possibility of drug-induced QT shortening has also been previously recognized (Schimpf et al., 2012; Huang et al., 2019).
However, drug-induced short QT syndrome is rare. Remdesivir chronically increased $I_{\text{hERG}}$ at concentrations that may be above clinical concentrations (Tempestilli et al., 2020). On the other hand, whether remdesivir can counteract against chloroquine or hydroxychloroquine-induced $I_{\text{hERG}}$ inhibition remains to be an important question that may warrant future basic and clinical investigations.

In addition to $I_{\text{Kr}}$ encoded by hERG, $I_{\text{Ks}}$ encoded by KCNQ plus KCNE1 is the other important potassium current responsible for cardiac repolarization. We also examined the effects of chloroquine, hydroxychloroquine, azithromycin and remdesivir on KCNQ+KCNE1 channels. Our data showed that none of the four drugs affect $I_{\text{KCNQ1+KCNE1}}$ acutely or chronically (Fig. 6 and 7).

Overall, out of the COVID-19 drugs examined in our study, at clinically relevant levels, chloroquine acutely caused significant decrease in $I_{\text{hERG}}$. Although hydroxychloroquine also blocks hERG, it is 8-fold less potent than chloroquine, and block may only occur at concentrations above those observed in patient serum. These data are in line with a recent study showing that hydroxychloroquine is safe for COVID-19 and not associated with a risk of ventricular arrhythmia due to drug-induced QTc interval prolongation (Sogut et al., 2020). Our data also revealed that $I_{\text{hERG}}$ was chronically increased by remdesivir at concentrations that may be beyond clinically relevant levels.

It remains to be determined whether QT-prolonging drugs are more likely to induce TdP arrhythmia in COVID-19 patients. Hospitalized COVID-19 patients may suffer from hypoxia, fever, electrolyte abnormalities especially hypokalemia, and viral- or autoimmune-induced myocardial injury (Hu et al., 2020a; Zeng et al., 2020; Clerkin et al., 2020). We have previously demonstrated that hypoxia, fever, hypokalemia, and protease activation can damage or facilitate
the damage of hERG function (Lamothe et al., 2017; Lamothe et al., 2016; Guo et al., 2009; Zhao et al., 2016). In fact, while hospitalized COVID-19 patients display respiratory symptoms such as shortness of breath, they also display cardiac arrhythmias (Wang et al., 2020; Du et al., 2020). In addition, COVID-19 patients receiving chloroquine or hydroxychloroquine as a therapy may respond to other medications differently because chloroquine and hydroxychloroquine are known to inhibit cytochrome P450 (CYP) enzymes (Rendic and Guengerich, 2020). CYPs are responsible for the metabolism of most foreign substances including 70–80% of clinically used drugs (Zanger and Schwab, 2013), and co-administration of CYP-inhibiting drugs may increase plasma levels of other drugs that inhibit I_{hERG}, enhancing the risk of drug-induced LQTS (Furst et al., 2002). Besides K^+ current inhibition, drugs may be proarrhythmic through other mechanisms. For example, while it was reported that clinical levels of azithromycin neither blocks I_{hERG} in hERG-HEK cells nor prolongs QTc in anesthetized dogs (Thomsen et al., 2006), another study reported that acute treatment of azithromycin results in a concentration dependent block of Na^+ currents, whereas chronic azithromycin treatment increases sodium current SCN5A by 2-fold (Yang et al., 2017). Finally, COVID-19 patients with pre-existing inherited arrhythmia syndromes may be more likely to develop LQTS (Wu et al., 2020). Due to these reasons, attention should be paid on monitoring ECG recordings for COVID-19 patients to prevent and treat arrhythmias in a timely manner.

**Conflict of Interest:** The authors declare that they have no conflicts of interest with the contents of this article.

**Authorship Contributions:**
Participated in research design: Zhang

Conducted experiments: Szendrey, Guo, Li, and Yang

Performed data analysis: Szendrey, Guo, Li, and Yang

Wrote or contributed to the writing of the manuscript: Szendrey and Zhang
References


**FOOTNOTES**

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LEGENDS FOR FIGURES

Fig. 1. At a concentration of 10 μM, chloroquine (CQ) but not hydroxychloroquine (HCQ), azithromycin (AZ) or remdesivir (RDV) acutely blocks \( \text{i}_{\text{hERG}} \). Representative \( \text{i}_{\text{hERG}} \) recorded in control (black) and after drug application (red) with the voltage protocol shown above the current traces. \( \text{i}_{\text{hERG}} \) in the presence of drug was normalized to the control and summarized as box plots on the bottom. The square represents the mean, the line within the box represents the median, original data are shown as dots with maximal and minimal data indicated with bars. Each dotted line links the current amplitudes recorded before and after drug administration in the same cell. **\( P<0.01 \) compared with control.

Fig. 2. Concentration-dependent acute effects of chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ) and remdesivir (RDV) on \( \text{i}_{\text{hERG}} \). \( \text{i}_{\text{hERG}} \) was elicited using the protocol shown in Fig. 1. The peak tail current amplitude at each drug concentration was normalized to the control current. Data from 13-19 cells were summarized and plotted against drug concentrations. The concentration-response relationships were fitted to the Hill equation to obtain the concentration for half maximal inhibition (\( \text{IC}_{50} \)). While CQ and HCQ blocked \( \text{i}_{\text{hERG}} \) in a concentration dependent manner, neither AZ nor RDV affected \( \text{i}_{\text{hERG}} \) at 10 or 50 μM.

Fig. 3. Acute effects of chloroquine (CQ) on activation-voltage relationships of hERG channels. Family of \( \text{i}_{\text{hERG}} \) was recorded by the voltage protocol shown above the current traces. The pulse currents measured at the end of 4-s depolarizing steps and the tail currents measured upon the repolarization to -50 mV were plotted against depolarizing (activation) voltages. The tail current-voltage relationships were fitted to the Boltzmann function to obtain the voltage for half maximal channel activation (\( \text{V}_{1/2} \)) and slope factors.
Fig. 4. Chronic effects of chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ) and remdesivir (RDV) on $I_{hERG}$. (A) representative $hERG$ currents in control (CTL) cells and those cultured with 10 μM CQ, HCQ, AZ or RDV for 24 h. (B) box plots showing maximal tail currents upon -50 mV repolarization from hERG-HEK cells cultured in control or with 10 μM CQ, HCQ, AZ or RDV for 24 h. **$P<0.01$ compared with control. (C) tail current-voltage relationships fitted to the Boltzmann equation function of $I_{hERG}$ recorded from cells cultured in control or with 10 μM CQ, HCQ, AZ or RDV for 24 h. **$P<0.01$ compared with control at voltages >0 mV.

Fig. 5. Concentration-dependent chronic effects of remdesivir (RDV) on $I_{hERG}$ and hERG expression. (A) box plots showing maximal tail currents upon -50 mV repolarization from hERG-HEK cells cultured in control or with various concentrations of RDV for 24 h. *$P<0.05$, **$P<0.01$ compared with absence of drug. (B) Western blot showing hERG expression of hERG-HEK cells cultured in control (CTL) or with 10 μM CQ, HCQ, AZ or RDV for 24 h. n=4, **$P<0.01$ compared with control. (C) Western blot showing hERG expression of hERG-HEK cells cultured in the absence or presence of various concentrations of RDV for 24 h. n=4, **$P<0.01$ compared with absence of drug.

Fig. 6. Chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ) or remdesivir (RDV) does not acutely affect $I_{KCNQ1+KCNE1}$. Top: Representative $I_{KCNQ1+KCNE1}$ traces recorded with the shown voltage protocol before and after 10 μM CQ, HCQ, AZ or RDV. Bottom: Peak
current amplitudes in the presence of drug were normalized to the respective control (CTL, in the absence of drug) in the same cell, and plotted as relative (Rel) $I_{\text{KCNQ1+KCNE1}}$.

**Fig. 7.** Chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ) or remdesivir (RDV) does not chronically affect $I_{\text{KCNQ1+KCNE1}}$. $I_{\text{KCNQ1+KCNE1}}$ was recorded using the voltage protocol shown on the top-left of Fig. 4A. $I_{\text{KCNQ1+KCNE1}}$ was measured at the end of 4-s depolarizing step to 50 mV from KCNQ1+KCNE1-HEK cells cultured in control or with 10 μM CQ, HCQ, AZ or RDV for 24 h. Data are shown in box plots for various treatments.

**Fig. 8.** Summary of the acute and chronic effects of chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ) or remdesivir (RDV) on $I_{\text{hERG}}$ and $I_{\text{KCNQ1+KCNE1}}$. Upper-arrow: increase; Down-arrow: decrease; Horizontal-line: no effect.
Figure 3

Graphs showing the current-voltage relationships for hERG channels in control (CTL) and with CQ treatment.

- **hERG-pulse (nA):**
  - CTL: Black squares
  - CQ: Red circles

- **hERG-tail (nA):**
  - CTL: Black circles
  - CQ: Red circles

Voltage range from -60 to 60 mV.
Figure 4

A

CTL  CQ  HCQ  AZ  RDV

-80 mV -70  -50

0.5 nA

2 s

B

$I_{hERG\text{-}tail}$ (nA)

0.0  1.0  2.0  3.0

CTL  CQ  HCQ  AZ  RDV

Drugs (10 μM)

C

$I_{hERG\text{-}tail}$ (nA)

0.0  1.0  2.0  3.0

-60  -30  0  30  60

Voltage (mV)

**  *

*CTL, CQ, HCQ, AZ, RDV*
Figure 5

(A) Graph showing \( I_{\text{hERG}-\text{tail}} \) vs. RDV (\( \mu M \)).

(B) Western blot analysis with bands at 245, 180, 135, 100, 48, and 35 kDa for hERG.

(C) Western blot analysis with bands at 155 kDa and 135 kDa for hERG and actin respectively.
Figure 7

[Box plots showing the distribution of KCNQ1+KCNE1 current (nA) for different drugs (10 μM).]

Drugs (10 μM):
- CTL
- CQ
- HCQ
- AZ
- RDV
Figure 8

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